

PHOTOREVERSIBLY PHOTOCHROMIC PIGMENTS FROM
BLUE-GREEN ALGAE (CYANOBACTERIA)

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by

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Academical dissertation

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Abstract Experiments on photomorphogenesis and chromatic adaptation of blue-green algae(cyanobacteria) indicate that at least one photoreversibly photochromic pigment is involved in light perception. Attempts were therefore made to get more direct evidence for the existence of such pigments. Extracts from different blue-green algae were electrofocused and/or gel-filtrated on Sephadex G-200, and fractions were tested spectrophotometrically. Four such pigments, phycochromes a, b, c and d with different spectral properties were found. Phycochrome a was found in the phycocyanin fraction while phycochromes c and d were found in the allophycocyanin fractions. None of these phycochromes could be isolated. Phycochrome b, however, was isolated and seems to be the α-subunit of phycoerythrocyanin. Absorbance difference spectra and action spectra for the light-induced in vitro conversions were measured. For phycochrome b also in vivo spectra were determined. The spectral properties of the different phycochromes are discussed with respect to their possible participation in the responses of the blue-green algae to light.			
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INTRODUCTION

The organisms traditionally called blue-green algae or cyanophytes have been proposed (Stanier et al. 1978) to be placed among the bacteria and renamed blue-green bacteria or cyanobacteria because of the prokaryotic structure of their cells.

According to Stanier and Cohen-Bazire (1977) "the cyanobacteria can be defined as organisms that harbor, within a prokaryotic cell, a photosynthetic apparatus remarkably similar in functional, structural, and molecular respects to that contained in the eukaryotic chloroplast".

As all algae and higher plants the blue-green algae possess the ability to evolve oxygen during photosynthesis and like these organisms they also contain chlorophyll a. Their major accessory pigments are the phycobiliproteins, and in this respect they resemble some eukaryotic algae (the rhodophytes and the cryptophytes). In the rhodophytes and blue-green algae these phycobiliproteins are arranged in special aggregates called phycobilisomes, which are located on the stroma side of the photosynthetic lamellae (Gantt and Conti 1966, 1969).

There are different kinds of phycobiliproteins in the blue-green algae. All blue-green algae seem to synthesize phycocyanin ($\lambda_{\max} \approx 620 \text{ nm}$)¹⁾, allophycocyanin ($\lambda_{\max} \approx 650 \text{ nm}$) and, according to Rippka et al. (1979), allophycocyanin B ($\lambda_{\max} \approx 670 \text{ nm}$). Many blue-green algae also synthesize different phycoerythrins ($\lambda_{\max} \approx 520\text{--}580 \text{ nm}$). In some blue-green algae still another kind of phycobiliprotein can be found, namely phycoerythrocyanin ($\lambda_{\max} \approx 570 \text{ nm}$) (Bryant et al. 1976).

In some of the algae which are able to synthesize phycoerythrins, the synthesis is influenced by the quality of the prevailing light. Among these algae some strains are capable of regulating

1) $\lambda_{\max}$ = wavelength of long-wavelength absorption band



the amount of phycoerythrin only, while others can regulate both the amount of phycoerythrin and phycocyanin (Tandeau de Marsac 1977). Members of this last group accumulate those pigments which are complementary in colour to the light under which they are grown. The regulation of pigment synthesis in a way that maximizes light absorption and gives the alga a colour complementary to that of the prevailing light is called complementary chromatic adaptation.

The first to observe these colour changes was Gaidukov (1902). He noted that the blue-green alga, *Oscillatoria santa*, became red when it was grown in green light and blue-green when the prevailing light was orange.

Kylin (1912) and later Boresch (1919, 1921) were aware that such colour changes were due to changes in the relative proportions of red and blue phycobiliproteins.

The participation of the accessory pigments in photosynthesis was pointed out for the first time when Engelmann (1883) irradiated eukaryotic and blue-green algae with light of different wavelengths and estimated oxygen evolution by means of aerotactic bacteria. Referring to these experiments Gaidukov (1902) writes: "Für grüne Zellen war das rothe Licht, für rothe das grüne, für blaugrüne das gelbe, für gelbe das blaugrüne Licht relativ am wirksamsten".

By measuring the quantum yield of photosynthesis as a function of wavelength Emerson and Lewis (1942) showed that phycocyanin in the blue-green alga, *Chroococcus*, is active in photosynthesis and that the light absorbed by phycocyanin is used almost as efficiently as the light absorbed by chlorophyll.

Action spectra of photosynthesis in different algae, especially red algae, made by Haxo and Blinks (1950) show that light absorbed by phycoerythrin and phycocyanin is used in photosynthesis and that these phycobiliproteins are the major light-harvesting pigments.

At about the same time French and Young (1952) studied the red alga *Porphyridium cruentum* and observed fluorescence from chlorophyll a with exciting light predominantly absorbed by the accessory pigments, phycoerythrin and phycocyanin.

After these findings it was evident that the capability among many blue-green algae to adjust the amount of phycoerythrin and sometimes also phycocyanin according to the quality of the prevailing light is a most efficient way of making maximum use of the available light energy.

Fujita and Hattori (1962) made one of the first attempts to explain the mechanism of the complementary chromatic adaptation. They determined action spectra for induction and inhibition of phycoerythrin synthesis in the blue-green alga, *Tolypothrix tenuis*, and interpreted the spectra as action spectra for conversion of a phycocyanin precursor to a phycoerythrin precursor and vice versa.

Diakoff and Scheibe (1973) repeated these measurements with the same alga and better equipment, giving better wavelength resolution but essentially the same result.

Also the action spectra for induction and inhibition of phycoerythrin synthesis in another blue-green alga, *Fremyella diplosiphon*, measured by Vogelmann and Scheibe (1978), are similar.

Haury and Bogorad (1977) made action spectra for synthesis of phycoerythrin and phycocyanin in *Fremyella diplosiphon*, but in a different way. They obtained spectra with about the same maxima in the red and green regions of the spectrum as the others, but in addition their spectra have maxima in the blue region.

In the same year as Fujita and Hattori made their action spectra for photoinduced changes of the content of phycobiliproteins in *Tolypothrix tenuis*, Lazaroff and Schiff (1962) studied photo-morphogenesis in another blue-green alga, *Nostoc muscorum* A.

Their action spectrum for the formation of filaments from a non-filamentous or aseriate stage has a maximum at about the same wavelength (650 nm) as the action spectra for inhibition of phycoerythrin synthesis mentioned earlier. The shape of the action spectrum for the photoreversal is, however, different from the action spectra for induction of phycoerythrin synthesis, with a very broad action band in the green region of the spectrum (Lazaroff 1966).

The action spectrum for induction of "motility" in *Nostoc commune* has a maximum at about 640 nm, and the corresponding reversal spectrum a maximum at about 520 nm (Robinson and Miller 1970). They also showed that this process was repeatedly photo-reversible. The same holds for the light-responses in *Fremyella diplosiphon* (Diakoff and Scheibe 1975).

If the latter alga is grown heterotrophically (in the dark), a brief daily irradiation (5 minutes) with red light promotes growth as well as total phycobiliprotein and chlorophyll content. A brief daily irradiation (5 minutes) with green light, however, depresses growth and the content of total phycobiliproteins and chlorophyll as compared to the treatment with red light. Green light given immediately after the red reverses the effect of the red light and vice versa. With brief light treatments this response was shown to be repeatedly photoreversible and the growth rate dependent on the quality of the last irradiation only.

In Table 1, a modified scheme after Vogelmann and Scheibe (1978), all the experiments mentioned have been collected to show the action maxima. In a review article by L.O. Björn (1979) all these action spectra are assembled in one figure.

In all the photoinducible changes mentioned, green light affects the algae in one way and this effect is reversed by red light. In some cases, mentioned earlier, it has also been shown that in experiments with alternating treatments with green and red light, it is always the last irradiation that determines the response.

Table 1

Action maxima (nm)						
		green-absorbing form		red-absorbing form		
Tolypothrix tenuis		541		641		Fujita & Hattori
" "	350	550	360	660		Diakoff & Scheibe
Fremyella diplosiphon		540	360	650		Vogelmann & Scheibe
" "	387	550	463	641		Haury & Bogorad
Nostoc muscorum A		530		650		Lazaroff & Schiff
Nostoc commune		520		640		Robinson & Miller

According to these findings it is now accepted that there must be at least one reversibly photochromic pigment controlling photomorphogenesis and chromatic adaptation in the blue-green algae - a pigment analogous to phytochrome but with a green - red antagonism instead of a red - far-red as in the case of phytochrome.

Scheibe (1972) extracted a reversibly photochromic pigment from *Tolypothrix tenuis* by repeated freezing and thawing. After centrifugation and treatment of the supernatant with ammonium sulphate, the fraction obtained was chromatographed on brushite to separate the photochromic pigment from phycoerythrin and phycocyanin. Allophycocyanin, however, was still present in the photochromic fraction.

The purpose of the present investigation was to find out if photoreversibly photochromic pigments could be obtained from other blue-green algae, if other separation techniques (iso-electric focusing and gel-filtration) could be used, and to determine some properties of the pigments. Emphasis has been placed on action spectra for pigment conversions, as they provide a means for comparing the extracted pigments with the physiological light-sensor involved in photomorphogenesis and complementary chromatic adaptation.

The thesis is based on the following publications:

- I Björn, G.S. & Björn, L.O. 1976. Photochromic pigments from blue-green algae: phycochromes a, b, and c. - *Physiol. Plant.* 36: 297-302. (The discussion in this publication is excluded and replaced by discussion in this dissertation.)
- II Björn, G.S. 1978. Phycochrome d, a new photochromic pigment from the blue-green alga *Tolypothrix distorta*. - *Physiol. Plant.* 42: 321-323.
- III Björn, G.S. & Björn, L.O. 1978. Action spectra for conversions of phycochrome c from *Nostoc muscorum*. - *Physiol. Plant.* 43: 195-200.
- IV Björn, G.S. 1979. Action spectra for in vivo and in vitro conversions of phycochrome b, a reversibly photochromic pigment in a blue-green alga, and its separation from other pigments. - *Physiol. Plant.* 46: 281-286.
- V Björn, G.S. 1980. Phycochromes b and d: their occurrence in some phycoerythrocyanin-containing blue-green algae (cyanobacteria). - *Physiol. Plant.* 48: 483-485.
- VI Results printed for the first time in this dissertation.

EXPERIMENTAL PROCEDURE

1. Algal strains and cultivation

Most of the algal strains used as well as their cultivation are described in I: Table 1. The other strains and their cultivation are described in V.

2. Extraction and separation of pigments

The algae were harvested by centrifugation, washed twice and resuspended in 0.01 M phosphate buffer ($\text{KH}_2\text{PO}_4/\text{Na}_2\text{HPO}_4$), pH 7,

or 0.01 M acetate buffer pH 4.4 at 4°C or, when phycobilisomes were isolated, in 0.75 M potassium phosphate buffer, pH 6.8, at 20°C.

The algal cells were broken by sonication and/or French press treatment. The preparations of phycobilisomes were made as described by Gantt et al. (1979); otherwise the suspensions were centrifuged at 10 000 x g for 10 minutes and the supernatant subjected to a renewed centrifugation at 80 000 x g for 1 h. The supernatant from the last centrifugation was dialysed overnight against deionized water and then put on an electrofocusing column (with 1-3 % Ampholine, pH 4-6, stabilized by a sucrose density gradient), and/or on a column of Sephadex G-200, equilibrated with 0.01 M phosphate buffer ($\text{KH}_2\text{PO}_4/\text{Na}_2\text{HPO}_4$) pH 7.

3. Spectrophotometry

The spectrophotometer used in all experiments was an Aminco DW-2. The instrument was operated in either the split-beam or the dual wavelength mode.

The source of actinic light and its arrangement as well as the instrument for measuring the actinic light are described in I.

RESULTS

I Photochromic pigments from blue-green algae: phycochromes a, b, and c.

Three types of photoreversibly photochromic pigments were found: phycochrome a in several blue-green algae, phycochrome b in *Tolypothrix distorta* and phycochrome c in *Nostoc muscorum* A and *Tolypothrix tenuis*.

Absorbance difference spectra for the different phycochromes were measured. For phycochrome a this spectrum was found to have a maximum at 630 nm and a minimum between 540 and 580 nm; when

the difference between 520 nm-irradiated and 630 nm-irradiated samples was measured.

The corresponding values for phycochrome b were 570 and 500 nm, when the absorbance difference between 500 nm-irradiated and 570 nm-irradiated samples was measured.

In the absorbance difference between the 520 nm-irradiated and the 650 nm-irradiated forms of phycochrome c there is one sharp maximum at 650 nm, while the absorbance change in the green-yellow region of the spectrum is very small and broad.

Transformation kinetics of phycochrome a from *Phormidium luridum* show that the initial reactions in both directions were of first order. Calculations for transformation cross sections and rate constants for the different phycochromes were also made.

The absorbance difference between the 520 nm-irradiated and the 630 nm-irradiated forms of phycochrome a is compared to the absorption difference between the f- and s- chromophores of phycocyanin, computed from data obtained by Vernotte (1971). Because of the great similarity between these spectra it was thought that the observed changes of phycochrome a reflect transformations between s- and f-type chromophores.

Based on the molar absorption coefficient for phycocyanin, determined at the absorption maximum, 618 nm (Vernotte 1971), and on our own measurements of the rate constants and the photon flux densities, it was possible to estimate the quantum yields for the transformations between the two forms of phycochrome a.

The quantum yields of transformations between the different forms of phycochrome b and c cannot be calculated, since the molar absorption coefficients are missing. Measurements of conversion rates made in light corresponding to the isosbestic point for phycochrome b indicate that the quantum yield for transformation of the short wavelength to the long wavelength form is about 2.4 times the quantum yield for the reverse transformation.

For phycochrome c the quantum yield for transformation from the short wavelength to the long wavelength form seems to be much higher than the corresponding value for the reverse transformation, since the absorbance change is so small in the green spectral region and the values of transformation cross section are about the same for both transformations. (The transformation cross section is the product of the absorption cross section and the quantum yield.)

Errata in I

	Wrong	Right
Page 301, Table 2	$f = \lambda/h \cdot c \cdot N$	$f = i \cdot \lambda/h \cdot c \cdot N$
	14.3×10^{-5}	1.43×10^{-5}
	14.0×10^{-5}	1.40×10^{-5}
	a total absorbance of 0.5 cm of sample (1 cm)	a total absorbance of sample (1 cm)
	ϵ = molecular absorption coefficient	ϵ = molar absorption coefficient
Col. 1, Line 5	isobestic	isosbestic
" 19	"	"
Col. 2, " 7	Tolypothrix distorta	Tolypothrix tenuis
" 13	phycocyanin c	phycochrome c
" 16	$1.8 \times 10^2 \text{ m}^2 \cdot \text{E}^{-1}$	$1.4 \times 10^2 \text{ m}^2 \cdot \text{mol}^{-1}$
Page 302		
Col. 1, Line 14	$5.9 \times 10^2 \text{ m}^2 \cdot \text{E}^{-1}$	$3.3 \times 10^2 \text{ m}^2 \cdot \text{mol}^{-1}$

II Phycochrome d, a new photochromic pigment from the blue-green alga *Tolypothrix distorta*.

A fourth photoreversibly photochromic pigment, phycochrome d, with a maximum absorbance increase at 650 nm caused by irradiation with 650 nm light and a corresponding decrease after irradiation with 610-620 nm light is described.

These absorbance changes can only be explained by postulating the existence of a minimum of three pigment forms. The conversions between two of them are supposed to be light dependent while the formation of the third form is thought not to be directly dependent on light.

The absorbance difference spectrum as well as action spectra for the conversions were measured.

Phycochrome d was found in the allophycocyanin fraction, and this fraction was compared to fractions containing allophycocyanin from two other blue-green algae, *Nostoc muscorum* A and *Phormidium luridum* with respect to photochromic behaviour.

While the allophycocyanin fraction from *Tolypothrix distorta* showed phycochrome d activity with an absorbance increase (maximum at 650 nm) when irradiated with 650 nm light, the corresponding fraction from *Nostoc muscorum* A showed phycochrome c activity with an absorbance decrease (minimum at 650 nm) when irradiated with 650 nm light. In the allophycocyanin fraction from *Phormidium luridum*, however, no photochromism could be observed.

III Action spectra for conversions of phycochrome c from *Nostoc muscorum*.

In paper I it was shown that the absorbance difference between green-irradiated and red-irradiated forms of phycochrome c has a maximum at 650 nm. In this paper the action spectra for the

in vitro conversions of the different forms were measured and it was found that the action in the red region of the spectrum has its maximum at 630 nm instead of at 650 nm.

After short irradiations of the pigment it was observed that the absorbance changed both during the light treatment and during the following dark period. The change during the dark period must depend on the disappearance or formation of an intermediate. Therefore the absorbance change from the start to the end of the light pulse and the total (light + dark) change was determined separately for various actinic wavelengths. By combining the values of the change from the start to the end of the light pulse, a spectrum of the absorbance change during the 630 nm light pulse could be constructed. A spectrum of the total change resulted from a combination of the values of the light + dark change. In these ways approximations were obtained for the absorption of two red-absorbing forms, P_{630}^C and P_{650}^C .

Transformation kinetics for phycochrome c in both directions were shown to be of first order, at least initially.

The photostationary state spectrum obtained by measuring absorbance changes by saturating irradiations with light of different wavelengths was determined and the great similarity to the in vivo action difference spectrum, which can be calculated from the action spectra for in vivo conversions (Lazaroff 1966), was pointed out.

IV Action spectra for in vivo and in vitro conversions of phycochrome b, a reversibly photochromic pigment in a blue-green alga, and its separation from other pigments.

Absorbance difference spectra as well as action spectra for transformations between the two forms of phycochrome b were measured both in vivo and in vitro.

The in vivo and in vitro spectra are very similar to each other,

indicating that the spectral properties of the pigment are not changed during preparation.

There is also good agreement between the in vitro absorbance difference spectrum and the difference between the in vitro action spectra (a weighing factor, supposedly corresponding to the ratio of quantum yields, was applied before subtraction). It seems therefore reasonable to believe that there are only two spectral forms of phycochrome b.

The kinetics for transformations between these forms of phycochrome b were also calculated and the initial reactions in both directions were found to be first order reactions.

The absorbance changes were shown to be accompanied by changes in fluorescence, and separation of phycobilisomes showed that phycochrome b is located in these particles.

V Phycochromes b and d: their occurrence in some phycoerythrocyanin-containing blue-green algae (cyanobacteria).

The occurrence among blue-green algae of phycochromes b and d, previously extracted from *Tolypothrix distorta*, was investigated. These photoreversibly photochromic pigments were found only in blue-green algae containing phycoerythrocyanin. Tests for their presence among blue-green algae lacking phycoerythrocyanin were negative.

It seems very likely that the absorbance changes observed in phycochrome b are due to changes in the α -subunit of phycoerythrocyanin. If this is the case this subunit can be regarded as phycochrome b.

VI Further results and discussion concerning phycochromes a and c (not published elsewhere).

Phycochrome a has been found in the phycocyanin fractions from all blue-green algae investigated so far. It has, like all the other phycochromes, been found in the phycobilisomes.

Phycochrome a from *Phormidium luridum*, prepared as described in paper I, was further investigated. When this phycochrome is irradiated with green light ($\lambda = 520$ nm), there is an absorbance decrease in the red region of the spectrum ($\lambda_{\text{max}} \approx 630$ nm) and this decrease is still more pronounced if the pigment is irradiated with red light ($\lambda = 630$ nm).

The absorbance decreased in this way can, by irradiation with green light, be restored to the level obtained after the first irradiation with green light. Between these two last mentioned absorbance values there is complete photoreversibility. If the pigment after irradiation with green light is left in darkness for 1-2 hours, the absorbance increases to the initial value measured before any irradiation. This dark conversion also occurs after irradiation with red light but the time needed for the corresponding conversion is much longer. Figure 1 shows these absorbance differences before and after irradiations with red and green light.

In Figure 2 the action spectra for the two photoreversible conversions are shown. For determination of these spectra, the sample, buffered to pH 8, was brought to one of the two end-points by irradiation for 3 min with either 520 or 630 nm light and thereafter irradiated with light of different wavelengths. The durations of the latter irradiations were chosen so that the conversions were within the initial phases, where the conversions are first order reactions (see I).

Since the action peaks coincide with the peaks in the absorption spectra of the different forms (as judged from the absorbance difference spectrum) only two forms of phycochrome a can be

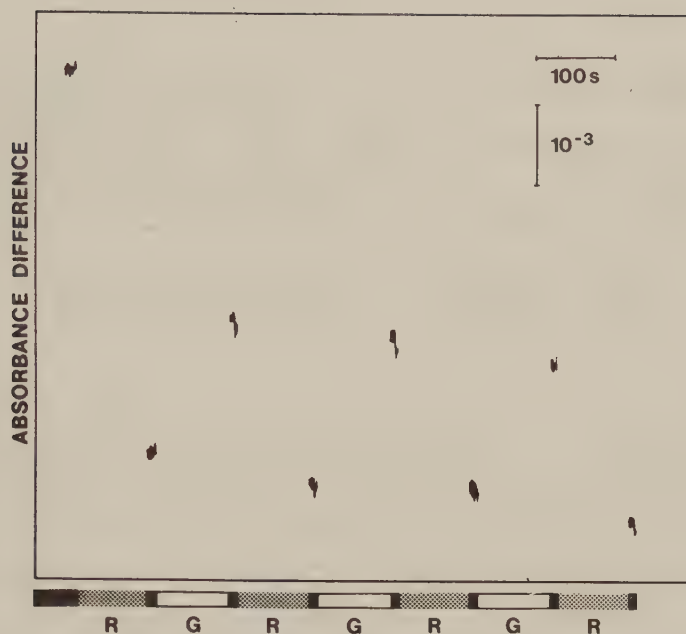


Figure 1. Light-induced absorbance changes in a phycocyanin fraction, pH 8. The sample was irradiated for 90 s alternatingly with 630 nm light (R) and 520 nm light (G). During the recording the sample was kept in darkness (black segments). Ordinate: Absorbance difference ($A_{630} - A_{520}$). Abscissa: Time.

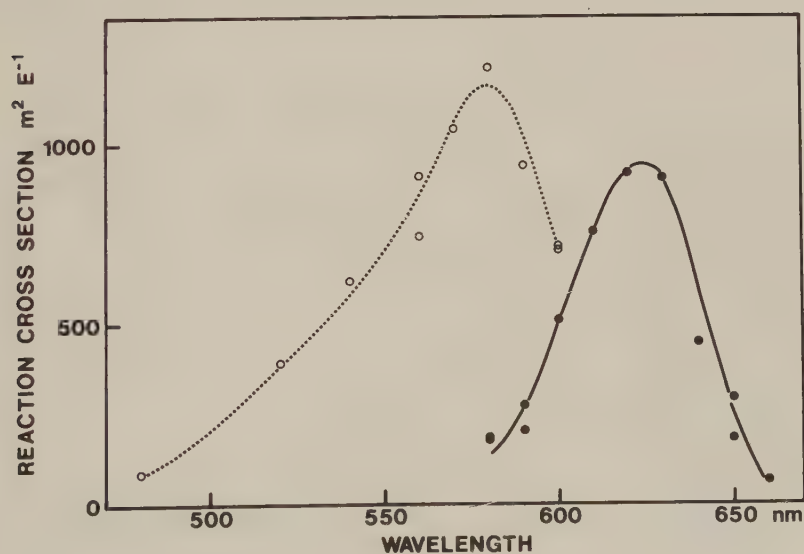
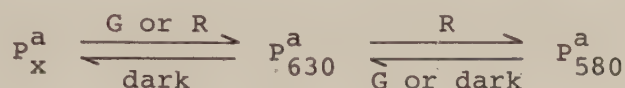


Figure 2. Action spectra for light-induced conversions of phycochrome a. Open circles: $p_{580}^a \rightarrow p_{630}^a$, solid circles: $p_{630}^a \rightarrow p_{580}^a$.

clearly distinguished. However, the dark conversion mentioned earlier indicates that there could be three forms, two forms which are interconvertible by light, P_{580}^a and P_{630}^a , and a third form, P_x^a . The relations between these forms might be:



/G and R stand for green ($\lambda = 520$ nm) and red ($\lambda = 630$ nm) light, respectively./ The interpretation of the data in terms of this model is as follows: P_x^a is the initial form which can be transformed to P_{630}^a by green or red light. Further irradiation with red light gives P_{580}^a , which is converted to P_{630}^a in darkness or by green light. P_{630}^a has not been found to be converted to the initial form, P_x^a , by any kind of light, but it is slowly converted to this form in darkness.

Phycochrome a activity ²⁾ was inhibited both by heat and urea treatments. Since absorbance and photochromism both depend on pH (Figure 3, a and b, respectively) these treatments were performed at pH 7 and pH 8.

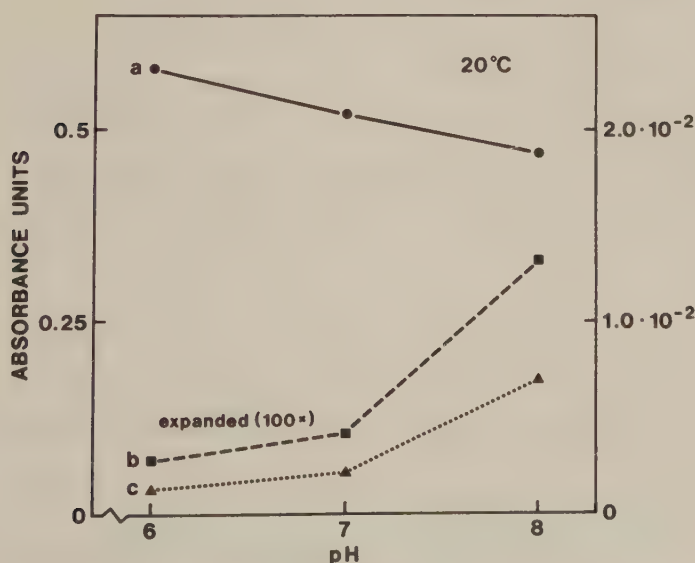


Figure 3. Influence of pH on maximum absorbance in the red spectral region of a phycocyanin fraction and on photochromism of this fraction. a: maximum "red" absorbance (absorbance units, left ordinate). b: maximum light-induced absorbance difference change, $\Delta(A_{630}-A_{520})$, (absorbance units, left ordinate). The values were multiplied by 100 before plotting. c: $\Delta(A_{630}-A_{520})$ divided by a (dimensionless, right ordinate). Abscissa: pH.

2) Activity denotes maximum photoinduced reversible absorbance change.

The heat treatment was carried out by immersing tubes with the pigment solution for 5 minutes in a water bath at different temperatures (30, 40, 50, 60, 70 and 80°C). Thereafter the solution was immediately cooled by immersing the tubes in water of room temperature (20°C) and absorption spectra (Figure 4 and 5) as well as phycochrome a activity (Figures 6 and 7) were measured.

The treatment with urea was carried out by adding an 8 M urea solution to the pigment solution to a final concentration of 6 M. Absorption spectra (Figure 8) and phycochrome a activity were measured when no further bleaching occurred.

In Figures 4 and 5 one can see that a pronounced absorbance decrease in the red spectral region occurs at 60°C, while a smaller increase occurs in the UV-A region of the spectrum. Compared to the original absorption in these spectral regions, the decrease is about twice as large as the increase. At 20°C, before heating, the absorption in the red region is about 7 times that in the UV-A region, while after heating to 60°C the absorption bands are of about equal strength. A further heating of the pigment solution to 80°C decreases the absorbance in the red region to about 14 % of the absorbance at 20°C, while the absorbance in the UV-A region is kept at the same level. After treatment at 80°C the maximum absorbance in the UV-A region is about twice that in the red region. As heating increases, the absorption maxima both in the red and UV-A region shift to shorter wavelengths.

In the red region of the spectrum, which originally has only one maximum at about 620 nm, treatment at 80°C causes the appearance of two maxima at about 585 and 635 nm (Figure 4, curve 5). This separation into two maxima is less pronounced at pH 8 but still more pronounced after treatment with urea (Figure 8). The absorbance decrease in the red spectral region at 60°C can be followed in curves a (Figures 6 and 7). Curves b in the same Figures show that there is no corresponding decrease in phycochrome a activity at this temperature. At pH 7 there is instead

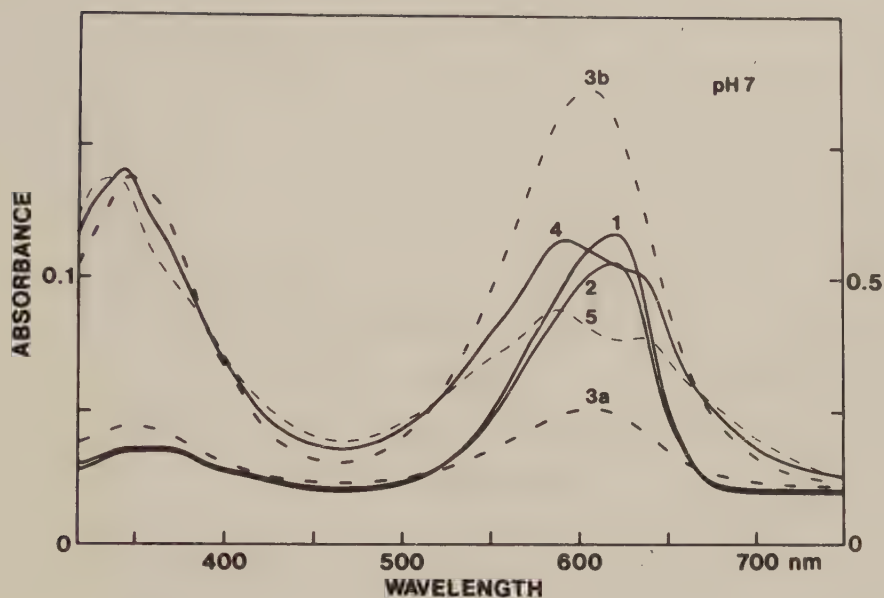


Figure 4. Absorption spectra of a phycocyanin fraction, pH 7, after different temperature treatments. 1, 30°C; 2, 50°C; 3a and b, 60°C; 4, 70°C; 5, 80°C. (1-3a, right ordinate; 3b-5, left ordinate.)

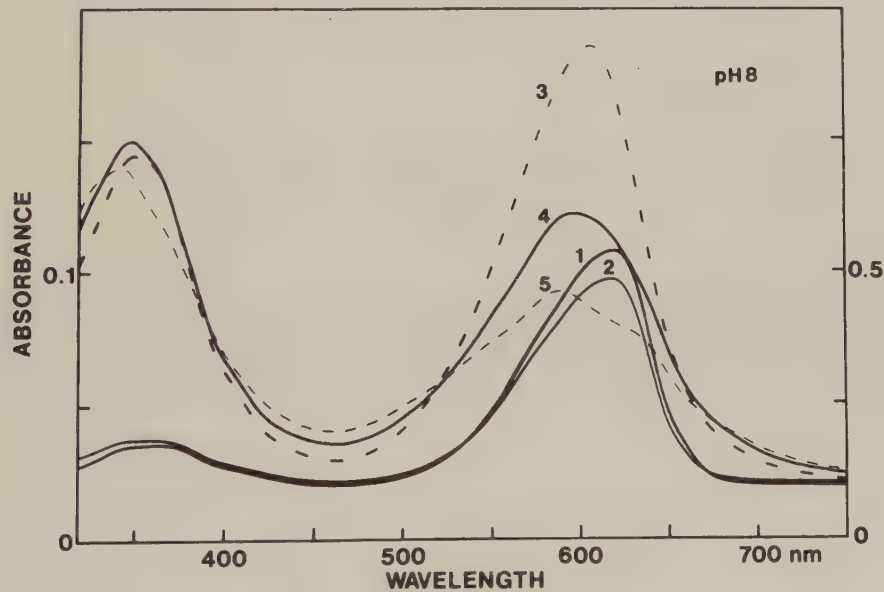
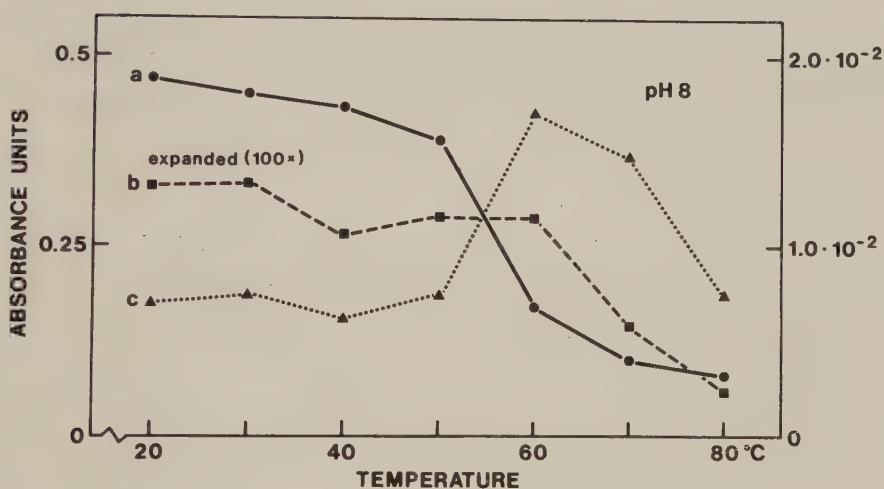
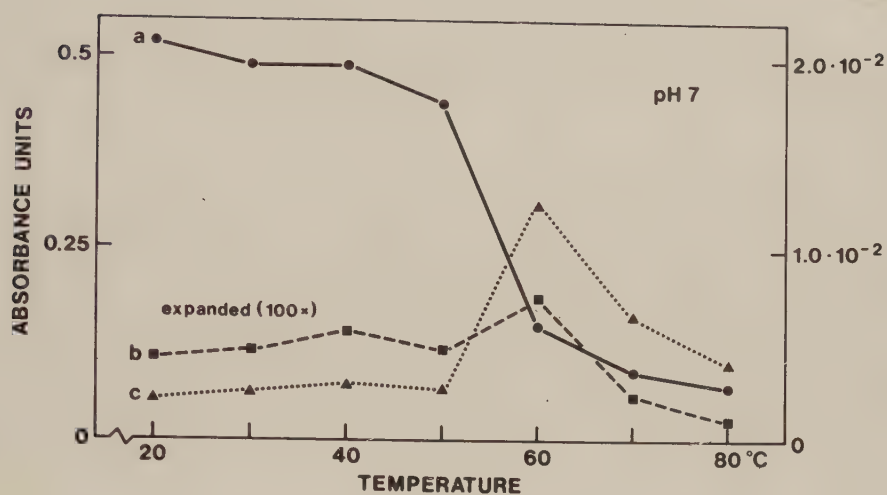


Figure 5. Absorption spectra of a phycocyanin fraction, pH 8, after different temperature treatments. 1, 30°C; 2, 50°C; 3, 60°C; 4, 70°C; 5, 80°C. (1 and 2, right ordinate; 3-5, left ordinate.)



Figures 6 (top) and 7. Influence of temperature on maximum absorbance in the red spectral region and on photochromism. Figure 6 refers to the same sample as Figure 4 and Figure 7 to the same one as in Figure 5. For explanation of a, b and c see text to Figure 3.

an increase in activity. The decrease in activity starts at 70°C.

The quotient (curves c in Figures 6 and 7) between phycochrome a activity (curves b) and maximum absorbance in the red spectral region (curves a) shows an increase at 60°C, while this quotient at 80°C is about the same as it is at 50°C or lower. A drastic decrease in this quotient occurs only when there is a change of absorption spectrum to a "two-peaked" spectrum (curves 4 and 5 in Figure 4 and curve 5 in Figure 5).

After the urea treatment the activity drops from $10.7 \cdot 10^{-4}$ to $4.3 \cdot 10^{-4}$ absorbance units at pH 7 and from $33.8 \cdot 10^{-4}$ to $2.4 \cdot 10^{-4}$ at pH 8. The absorbance decrease in the red spectral region is similar to the decrease obtained after heating to 80°C.

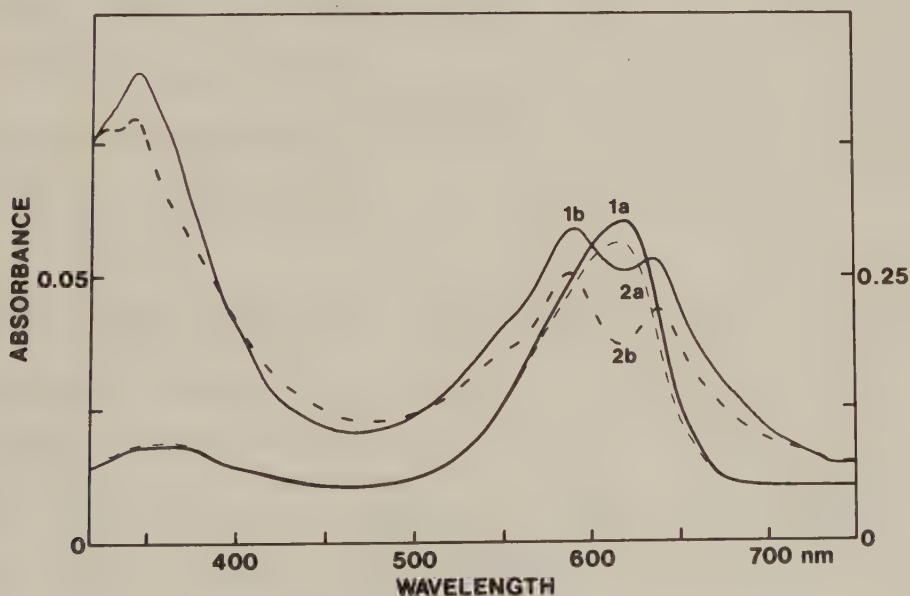


Figure 8. Absorption spectra of phycocyanin fractions before (a) and after (b) treatment with urea. 1, pH 7; 2, pH 8. (1a and 2a, right ordinate; 1b and 2b, left ordinate.)

As mentioned in paper I a great similarity was found in the absorbance difference between the 520 nm-irradiated and the 630 nm-irradiated forms of phycochrome a compared to the absorption difference between the f- and s-chromophores of phycocyanin, computed from Vernotte's data (1971). The action spectra (Figure 2) for the two photoreversible conversions have their action peaks at the same wavelengths as maxima and minima in the mentioned difference spectra. These values also appear in the "two-peaked" absorption spectra obtained when a phycocyanin fraction is heated to 80°C at pH 7 (Figure 4) or phycocyanin is treated with urea as mentioned earlier (Figure 8).

Since the agreement between the absorbance difference spectrum and the difference computed from the absorption of f- and s-chromophores of phycocyanin is so good, it was thought that the observed changes of phycochrome a reflect transformations between the two s- and f-chromophores in phycocyanin. However, since then it has been accepted that there are three chromophores (2 s- and 1 f-chromophores) bound to the protein of phycocyanin (Glazer et al. 1973). According to these findings it is more difficult to explain the observed photoreversible changes of phycochrome a. The changes observed in fractions containing phycocyanin are only a small part of the total absorbance, about 0.2 % at pH 7 and 0.7 % at pH 8.

Phycochrome c has earlier been found in fractions from *Nostoc muscorum* A and from the chromatically adapting blue-green alga *Tolypothrix tenuis*. The absorbance difference spectra (Figures in paper I) are different to some extent. While phycochrome c from *Nostoc muscorum* A has a very sharp negative band at 650 nm and a weak shoulder around 625 nm after irradiation with 650 nm light, the spectrum for the pigment from *Tolypothrix tenuis* has two peaks, one at about 625 nm and another at 650 nm. These two slightly different photoreversibly photochromic pigments are, however, both referred to as phycochrome c, since for both, absorbance at 650 nm decreases when they are irradiated with 650 nm light and the absorbance is restored by irradiation with 520 nm light.

Phycochrome c has now also been found in the allophycocyanin fractions from three other blue-green algae, *Nostoc muscorum* (Camb. 1453/12), *Fremyella diplosiphon* and *Nostoc* sp. strain Mac. The two last mentioned strains adapt chromatically and the absorbance difference spectra of their phycochrome c are very similar to each other and to the one obtained from *Tolypothrix tenuis*, while the spectra for phycochrome c from the two *Nostoc muscorum* strains are identical. There are three possible reasons for the discrepancy between the two groups of phycochrome c.

1. There is a mixture of phycochrome a and phycochrome c in the preparation from the chromatically adapting algae. This is a possibility since the fraction containing phycochrome c is located above the phycocyanin fraction containing phycochrome a in the electrofocusing column. These fractions are therefore difficult to separate when the column is emptied. When extracts from the *Nostoc muscorum* strains are electrofocused, however, the allophycocyanin fraction containing phycochrome c is located below any other fractions in the column.

Support for this interpretation comes from work done by Scheibe (1972), who after separating a photochromic pigment from *Tolypothrix tenuis* by another technique, obtained an absorbance difference spectrum similar to that measured in this laboratory for the *Nostoc muscorum* pigment, with maximum change at 650 nm and a shoulder around 625 nm.

2. The intermediate state investigated in paper III is more stable in the fraction obtained from the chromatically adapting strains, i.e. the dark conversion between P_{630}^C and P_{650}^C occurs more rapidly in phycochrome c from *Nostoc muscorum* than in phycochrome c from the chromatically adapting strains.

3. They are different pigments.

In paper I the quantum yield for transformation of phycochrome c from short- to long-wave form was supposed to be much higher than the corresponding value for the reverse transformation. By

taking the quotient between the reaction cross sections (determined in paper III) at the isosbestic point (560 nm, estimated from the absorbance difference spectra in paper I), the quotient between the quantum yields can be estimated to be about 20.

REMARKS CONCERNING PHYCOCHROME b

In paper I and paper IV two different values for the quotient between the quantum yields for the conversions of phycochrome b in both directions are given. In paper I the value is 2.4 and in paper IV 3.4. These values have been calculated in two different ways. In paper I the absorbance differences ($A_{570} - A_{510}$) were compared after saturating irradiations at 480, 570 and 525 nm. At the isosbestic point (525 nm) an equilibrium consisting of 70.5 % of the pigment in the longwave form and 29.5 % in the shortwave form was obtained (Figure 9 a and b). The quotient between these values gives 2.4.

The value used in paper IV, 3.4, was obtained by taking the quotient between the conversion cross sections (the ordinates of the action spectra) at the isosbestic point.

The values obtained by the two methods should be equal if there are only two forms of phycochrome b as has been proposed. The discrepancy cannot be explained at present.

CONCLUSIONS

From all the blue-green algae investigated one or more fractions containing photoreversibly photochromic pigments, phycochromes, have been extracted. These pigments seem to be very closely bound to or to be a part of at least some of the phycobili-proteins known to exist in blue-green algae.

Only one of these phycochromes, phycochrome b, has so far been shown to exist in vivo. This phycochrome has also been separated

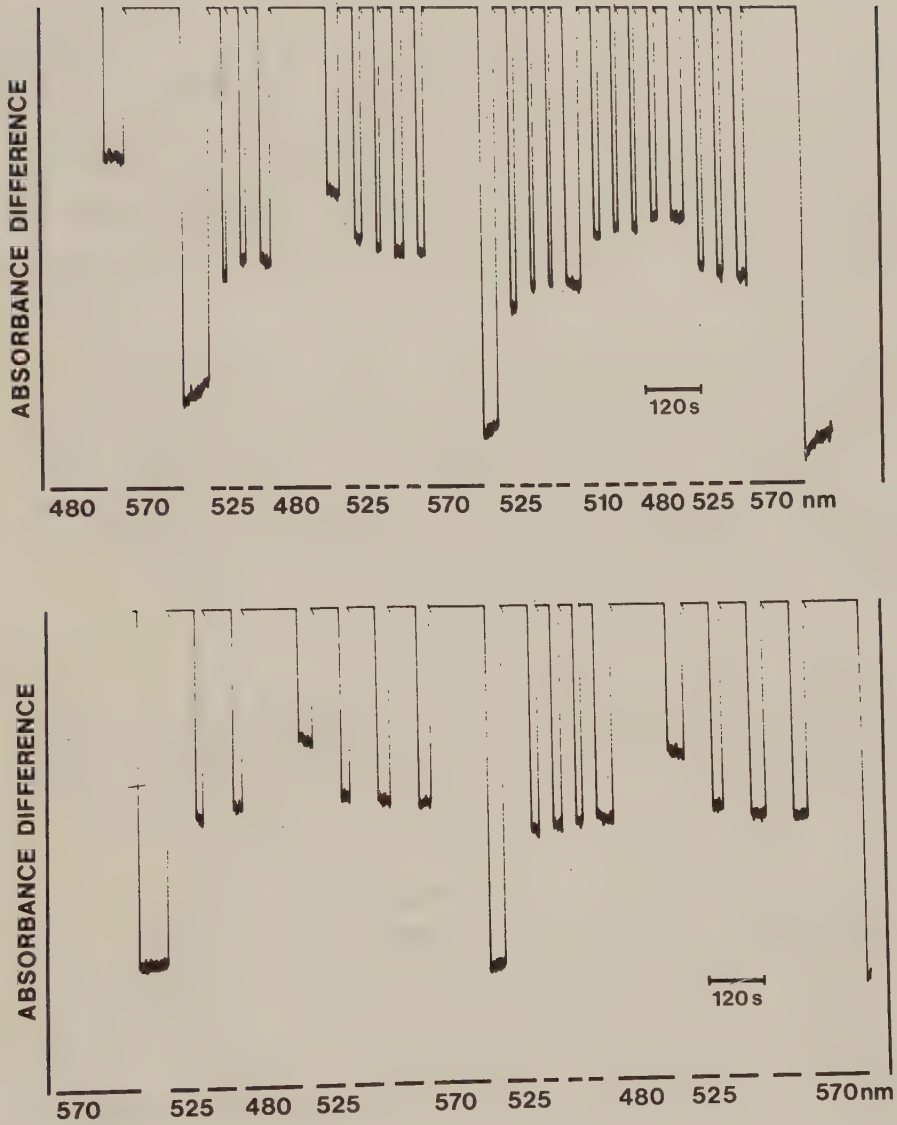


Figure 9 a and b. Light-induced absorbance changes of phycochrome b from *Tolypothrix distorta*. Ordinate: Absorbance difference ($A_{570}-A_{510}$). Abscissa: Time. The wavelengths and segments below the recordings indicate the duration of irradiation with light of the wavelengths indicated. Recording was made between the irradiations with the sample kept in darkness except for the measuring beams.



from the bulk phycobiliprotein and most likely found to correspond to the α -subunit of phycoerythrocyanin. Phycochromes c and d are found in the allophycocyanin fractions from different algae. Phycochrome a is discussed in VI in this dissertation. It has not been possible with the separation techniques used in this investigation to isolate phycochromes a, c and d from other pigments.

Since only a small part of the total absorbance of the samples at 650 nm is changing photoreversibly (about 4 % for phycochrome c and about 0.6 % for phycochrome d at pH 7) the observed absorbance changes must be due to changes in only some of the allophycocyanin molecules or in other molecules firmly bound to some of the allophycocyanin molecules.

Phycochrome c is the only one of the photoreversibly photochromic pigments investigated that shows some resemblance with in vivo action spectra measured for chromatic adaptation and for some other photoinduced effects in blue-green algae.

In vitro action spectra for phycochrome c from *Nostoc muscorum* A was, however, found to be different both from the absorbance difference spectrum and from in vivo action spectra (Lazaroff and Schiff 1962, Lazaroff 1966), while the photostationary state spectrum shows great similarity to the in vivo action difference spectrum, calculated from data obtained by Lazaroff (1966). A comparison between these spectra is shown by L.O. Björn (1979).

Since the in vivo and in vitro action spectra are not identical, the question whether phycochrome c is the photoreversible pigment responsible for the physiological response found in some blue-green algae can still not be answered.

The first part of the paper discusses the importance of the study and the objectives of the research. It also mentions the scope of the study and the limitations. The second part of the paper discusses the methodology used in the study. It includes the data collection methods and the data analysis methods. The third part of the paper discusses the results of the study. It includes the findings and the conclusions. The fourth part of the paper discusses the implications of the study. It includes the practical implications and the theoretical implications. The fifth part of the paper discusses the future research. It includes the suggestions for further research and the suggestions for further study.

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